Apolipoprotein A-II/A-I Ratio Is a Key Determinant in Vivo of HDL Concentration and Formation of Pre-β HDL Containing Apolipoprotein A-II

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ABSTRACT: Overexpression of human apolipoprotein A-II (apo A-II) in mice induced postprandial hypertriglyceridemia and marked reduction in plasma HDL concentration and particle size [Boisfer et al. (1999) *J. Biol. Chem. 274,* 11564−11572]. We presently compared lipoprotein metabolism in three transgenic lines displaying plasma concentrations of human apo A-II ranging from normal to 4 times higher, under ad libitum feeding and after an overnight fast. Fasting dramatically decreased VLDL and lowered circulating human apo A-II in transgenic mice; conversely, plasma HDL levels increased in all genotypes. The apo A-I content of HDL was inversely related to the expression of human apo A-II, probably reflecting displacement of apo A-I by an excess of apo A-II. Thus, the molar ratios of apo A-II/A-I in HDL were significantly higher in fed as compared with fasted animals of the same transgenic line, while endogenous LCAT activity concomitantly decreased. The number and size of HDL particles decreased in direct proportion to the level of human apo A-II expression. Apo A-II was abundantly present in all HDL particles, in contrast to apo A-I mainly present in large ones. Two novel findings were the presence of pre-β migrating HDL transporting only human apo A-II in the higher-expressing mice and the increase of plasma HDL concentrations by fasting in control and transgenic mice. These findings highlight the reciprocal modifications of VLDL and HDL induced by the feeding−fasting transition and the key role of the molar ratio of apo A-II/A-I as a determinant of HDL particle metabolism and pre-β HDL formation.

Plasma concentrations of high-density lipoproteins (HDL)[1] are inversely and independently correlated with risk for coronary artery disease (*1*−*3*). The protective effect of HDL has been attributed to their role in reverse cholesterol transport from extrahepatic tissues to the liver (*4*, *5*) and their ability to protect LDL against oxidation (*6*). Because low plasma HDL levels are often accompanied by hypertriglyceridemia, it is debated whether a deficiency of plasma HDL can exert a direct proatherogenic influence or whether HDL act as markers for the accompanying high levels of triglyceride (TG)-rich lipoproteins and their remnants. HDL particles are heterogeneous with respect to size and apolipoprotein composition, and it has been proposed from studies in transgenic mice that HDL carrying only apolipoprotein (apo) A-I have an antiatherogenic effect, in contrast to HDL transporting both apo A-I and apo A-II (*7*). Moreover, pre-β HDL, which are considered as the preferential acceptor of cholesterol upon efflux from peripheral tissues, have until now been shown to contain only apo A-I (*8*).

Elevated plasma HDL and apo A-I concentrations are not, however, predictive of protection against development of atherosclerotic lesions. Thus, mice overexpressing murine apo A-II develop aortic lesions on a chow diet, despite elevated plasma HDL levels with high apo A-I content (*9*). On the contrary, mice overexpressing human apo A-II have decreased plasma HDL levels but do not develop atherosclerotic lesions on chow (*10*). However, controversial results have been obtained with a high-fat and high-cholesterol diet: mice expressing human apo A-II at moderately elevated levels (43 mg/dL) were more protected than control mice against lesion development after 5 months on an atherogenic diet (*11*), while a different mouse line overexpressing human apo A-II at high levels (91−101 mg/dL) showed increased aortic lipid infiltrations relative to control mice after a 9 month administration of an atherogenic diet (*10*). Taken together, these observations suggest that the relationship between HDL, apo A-I, apo A-II, and atherogenesis is more complex than envisioned previously (*12*).

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[1] Abbreviations: apo, apolipoprotein; huAIItg, human apolipoprotein A-II transgenic; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; FC, free cholesterol; CE, cholesteryl ester; TC, total cholesterol; GGE, gradient gel electrophoresis; SDS, sodium dodecyl sulfate; FPLC, fast protein liquid chromatography; LCAT, lecithin cholesterol acyltransferase; HL, hepatic lipase; LPL, lipoprotein lipase; CETP, cholesterol ester transfer protein; PLTP, phospholipid transfer protein.

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We have recently established a mouse model overexpressing human apo A-II and which displays hypertriglyceridemia concomitantly with low plasma HDL in the fed state, such HDL being enriched in human apo A-II (*13*). This hypertriglyceridemia tended to normalize when transgenic mice were subjected to an overnight fast. Because an inverse relationship often exists between plasma triglyceride levels and HDL (*14*, *15*), we hypothesized that the decrease in VLDL may be accompanied by elevation in plasma HDL. This working hypothesis would be consistent with the contribution of surface components of triglyceride-rich lipoproteins to the formation of HDL, as first proposed by Tall and Small (*16*). On the other hand, studies by other laboratories in transgenic mice expressing apo A-II at levels up to 3 times the normal plasma concentration (*17*, *18*) have been performed mainly in fasted mice in which perturbation of lipoprotein metabolism is less marked than we observed in mice fed ad libitum (*13*). The aims of the present study were (i) to determine whether fasting would induce reciprocal changes in VLDL and HDL and (ii) to establish the influence of nutritional state and the level of apo A-II expression on lipoprotein metabolism and intravascular remodeling.

EXPERIMENTAL PROCEDURES

Generation of Transgenic Mice. Transgenic mice were generated by microinjection of the 3 kb genomic clone of the human apo A-II gene (−911/+2045) into one-cell embryos of (C57BL/6J × CBA/2J) F1 female mice (IFFA-CREDO, Lyon), as previously described (*13*). The three founder mice, β, δ, and λ, integrated approximately 7, 7, and 25 copies of the human apo A-II gene, respectively. They were backcrossed for at least 8 generations to strain C57BL/6J.

Animals. The animals were housed in animal rooms with alternating 12 h periods of light (7 a.m.−7 p.m.) and dark (7 p.m.−7 a.m.). All transgenic mice were hemizygous for the human apo A-II transgene and over 8 weeks of age. They were fed a chow diet (UAR, Villemoisson-sur-Orge), with free access to food and water, unless otherwise specified. Male and female transgenic mice were used in equal proportions in all studies. The presence of the human apo A-II transgene was determined by PCR of tail-derived DNA. Human apo A-II was also measured in plasma from all animals used in this study by immunonephelometry using an antibody (IMMUNO-AG) specific for human apo A-II and not recognizing mouse apo A-II. Blood was drawn from the retroorbital venous plexus or the abdominal vein between 9 and 12 a.m. Intravenous injections were administered into the jugular vein. For fasting mice, food was withdrawn at 6 p.m. Plasma samples were used fresh for all analyses.

Lipoprotein Analysis. Blood was collected into EDTA-containing tubes on ice. Plasma samples from 15−20 mice from each group were pooled, supplemented with 0.005% gentamycin/1 mM EDTA/0.04% sodium azide and protease inhibitors, and ultracentrifuged for lipoprotein isolation. Chylomicrons were first prepared by ultracentrifugation at 10000*g* for 30 min at 20 °C and washed once by ultracentrifugation for 18 h at 100000*g* and 10 °C to eliminate contamination by albumin. Then, sequential ultracentrifugations were performed at 100000*g* and 10 °C to isolate VLDL and LDL (for 18 h at densities of 1.006 and 1.063 g/mL, respectively), and HDL (for 40 h at a density of 1.21 g/mL). LDL and HDL were dialyzed against phosphate-buffered saline. Protein content of lipoproteins was measured according to Lowry et al. (*19*). Triglyceride (Biotrol A 01548), total cholesterol (Biotrol A 01368), free cholesterol (Biotrol A 01371), and phospholipid (BioMérieux PAP 150) contents were determined with commercial kits.

Fast protein liquid chromatography (FPLC) was performed on plasma pools of five mice from each group. Pooled plasma (0.2 mL) was chromatographed using two Superose 6 columns operating in series. The elution rate was 0.4 mL/min; 0.2 mL fractions were collected into 96-well microplates, and TG and total cholesterol contents were determined in each tube using commercial kits.

HDL Characterization by Analytical Ultracentrifugation. After an initial ultracentrifugation to eliminate chylomicrons (see above), 6 mL plasma pools from 10 control and 10 β transgenic mice or 9 mL plasma pools from 14 δ and 14 λ transgenic mice, either fed ad libitum or fasted for 16 h, were ultracentrifuged for 40 h at 1.21 g/mL to isolate lipoproteins. Analytical runs were then carried out on a MSE Centriscan 75 analytical ultracentrifuge operating in the refractometric mode at 550 nm. Details of such analyses and of the calculations used for interpretation of the traces obtained on the recorder have been previously published (*20*).

HDL Characterization by Density Gradient Ultracentrifugation. After an initial ultracentrifugation to eliminate chylomicrons, plasma pools from 10−12 control and β transgenic mice, and 16−23 δ and λ transgenic mice, either fed ad libitum or fasted for 16 h, were subjected to density gradient ultracentrifugation according to a previously published procedure (*21*). Briefly, a five-step discontinuous density gradient was constructed with saline (NaCl/NaBr) solutions of respective densities (from bottom of tube to top) 1.24, 1.21 (serum added with solid KBr), 1.063, 1.019, and 1.006 g/mL. The only noteworthy difference, compared with our original publication (*21*), was that, to accommodate the gradient into Beckman UltraClear tubes in place of the original cellulose nitrate ones, the volume of the upper solution was decreased from 3 to 2.5 mL. Such gradients were then centrifuged in a Beckman SW-41 swing-out rotor at 40 000 rpm for 44 h at 17 °C. Fractions (0.4 mL each) were subsequently recovered by aspiration and submitted to dialysis against PBS/EDTA/sodium azide, pH 7.4, for 3 × 12 h at 4 °C.

Size Distribution of HDL. The size distribution of total HDL isolated by sequential ultracentrifugation and of HDL subfractions prepared by density gradient ultracentrifugation was analyzed by nondenaturing gradient gel electrophoresis (GGE) in 4−20% polyacrylamide gels (pre-cast, BioRad). HDL were stained with Coomassie Brilliant Blue (R250). The HMW Calibration kit (Pharmacia−Amersham) was used for calibration of HDL particle size (*22*).

Nondenaturing Two-Dimensional Gel Electrophoresis. HDL prepared by sequential or density gradient ultracentrifugation were first electrophoresed in 0.75% (w/v) agarose (BioRad) in 50 mM barbital buffer (pH 8.6), at 5 mA/lane for 90 min (total migration 4 cm). In the second dimension, polyacrylamide gradient gel electrophoresis was carried out in a 8−25% precast gradient gel (Phast System, Pharmacia−Amersham), as described in ref *23* with slight modifications. The samples migrated at 300 V/h for 2 h. The migration

rate of individual lipoproteins during agarose electrophoresis was standardized with reference to plasma LDL, VLDL, and HDL visualized with Sudan black. To determine the migration patterns of purified apo A-I and A-II and to evaluate the particle size of HDL, we electrophoresed HDL, apo A-I, apo A-II, and HMW standards (Pharmacia−Amersham) first in agarose and then in 8−25% gels. However, all proteins migrated more rapidly than HDL in this system and were not detected in the second-dimension electrophoresis. Therefore, HDL were electrophoresed in agarose as described above, and HMW standards were added during the last 5 min of migration. The agarose strip containing both HDL and HMW standards was then electrophoresed in 8−25% gels as above. Purified apo A-I and A-II were also electrophoresed in agarose for 5 min and then in 8−25% gels as above.

Apolipoprotein Analysis. In lipoprotein fractions, human apo A-II was quantified by immunonephelometry (as above) and by ELISA in the low-expressing β mice. Apolipoproteins were analyzed by 15% SDS−PAGE (*24*) and 4−20% SDS−PAGE (pre-cast gels, BioRad), under nonreducing conditions in order to detect the dimeric form of human apo A-II. A constant amount of protein was loaded on each gel to compare relative VLDL or HDL apolipoprotein contents between transgenic and control mice. The gels were scanned using the NIH Image analysis program, and the percentages of the various apolipoproteins were calculated. Apo A-I and A-II were detected by Western blotting following SDS−PAGE, GGE, or two-dimensional gel electrophoresis and protein transfer to nitrocellulose membranes (0.45 or 0.22 μm, BioRad). Human apo A-II was detected by a rabbit anti-human apo A-II antiserum which does not recognize mouse apo A-II (courtesy of Dr. A. Mazur, INRA, Theix, France). The presence of mouse apo A-I and mouse apo A-II was determined by immunoblotting with specific rabbit antisera (Biodesign). Bands were visualized with an alkaline phosphatase substrate system (BioRad).

RNA Isolation and Northern Blot Analysis. Total RNA was isolated from the liver using the RNA Instapure kit (Eurogentec). RNA samples (15 μg) were separated in 3% formaldehyde-containing agarose gels and transferred to nylon membranes (Hybond N^+, Amersham Pharmacia Biotech). The membranes were hybridized to the human apo A-II cDNA probe. The 18S RNA probe was used as an internal standard. Autoradiograms were scanned and analyzed with the NIH Image programm.

LCAT Assays. Plasma endogenous LCAT activity was measured in individual control and transgenic mice by using the lipoproteins of the whole plasma as substrate, and results were expressed in nanomoles per milliliter per hour (*25*). Plasma exogenous LCAT activity was determined as previously described (*26*).

Statistical Analysis. Results are given as mean ± SE, and differences were determined using the *t*-test for nonpaired samples after analysis of variance, using GraphPad Prism.

RESULTS

Fasting Increases HDL and Lowers Human Apo A-II Concentration. Our previous study reported lipoprotein modifications induced on ad libitum feeding by a 2-fold (line δ) and a 4-fold (line λ) overexpression of human apo A-II (*13*). The marked hypertriglyceridemia and the presence of human apo A-II in the VLDL of our transgenic mice were at variance with the mild hypertriglyceridemia and the absence of apo A-II in the VLDL of transgenic mice overexpressing either murine (*27*) or human apo A-II (*18*). Because these latter studies were performed in fasted mice, we asked ourselves the question whether the differences between our study and those of Hedrick et al. (*27*) and Marzal-Casacuberta et al. (*18*) stemmed in part from the different nutritional states. Since the level of transgene expression may also influence the extent of lipoprotein modification, we added the transgenic line β in the present study, which expresses human apo A-II at the normal plasma concentration (20−30 mg/dL).

Upon analysis by FPLC, HDL was the major lipoprotein fraction in both control and β mice irrespective of nutritional state, whereas human apo A-II expression at higher levels markedly decreased HDL in δ and λ mice (Figure 1). On ad libitum feeding, VLDL levels increased proportionately to the expression of apo A-II, as reported (*13*). The elevated concentrations of VLDL were already present in β mice, in which a second peak of smaller HDL particles appeared. HDL were scarcely detectable in δ and λ mice, because of the higher scales of the corresponding figures. After an overnight fast, VLDL levels diminished drastically in all groups of mice, whereas remnant/LDL particles (fractions 35−62) increased and were enriched in cholesterol in λ mice.

Overnight fasting led to a decrease in the plasma concentration of human apo A-II, especially in λ mice (47%) (Table 1). Levels of all plasma lipids were equally reduced in transgenic mice, particularly TG, although a mild hypertriglyceridemia persisted in λ mice. Conversely, the low cholesteryl ester (CE)/total cholesterol (TC) ratio characteristic of fed transgenic mice increased to near-normal levels with the exception of λ mice. The decrease in plasma human apo A-II probably did not result from a lower transcription rate, because the amounts of human apo A-II mRNA in the liver, expressed as the ratios to 18S RNA, were comparable in fed and fasted transgenic mice (19.5 ± 2.8 and 24 ± 2 in 5 fed and 5 fasted δ mice, respectively; 21.2 ± 3.4 and 29 ± 4 in 5 fed and 5 fasted λ mice, respectively).

Tables 2 and 3 illustrate the chemical compositions of lipoproteins in fed and fasted animals, respectively. Four notable differences were induced by the overnight fast: (1) the disappearance of chylomicron-like large VLDL of transgenic mice (although approximately 10% of fasted λ mice still had chylomicrons), and the drastic reduction in VLDL (up to 9-fold); (2) the increase in the remnant/LDL fraction (up to 2.5-fold), which was relatively enriched in cholesterol; (3) the increase in HDL (up to 1.5-fold) in all groups of animals; and (4) the transport of apo A-II mainly by HDL, except in fasted λ mice in which a minor amount of apo A-II was also carried by TG-rich lipoproteins.

The CE/TC ratio in individual lipoproteins was not detectably different between HDL from control and transgenic mice, irrespective of their nutritional state. However, this ratio was lower in apo B containing lipoproteins, particularly in fed transgenic mice. Therefore, the very low CE/TC ratio in total plasma from fed transgenic mice (Table 1) may be partly attributable to the significant accumulation of TG-rich lipoproteins.

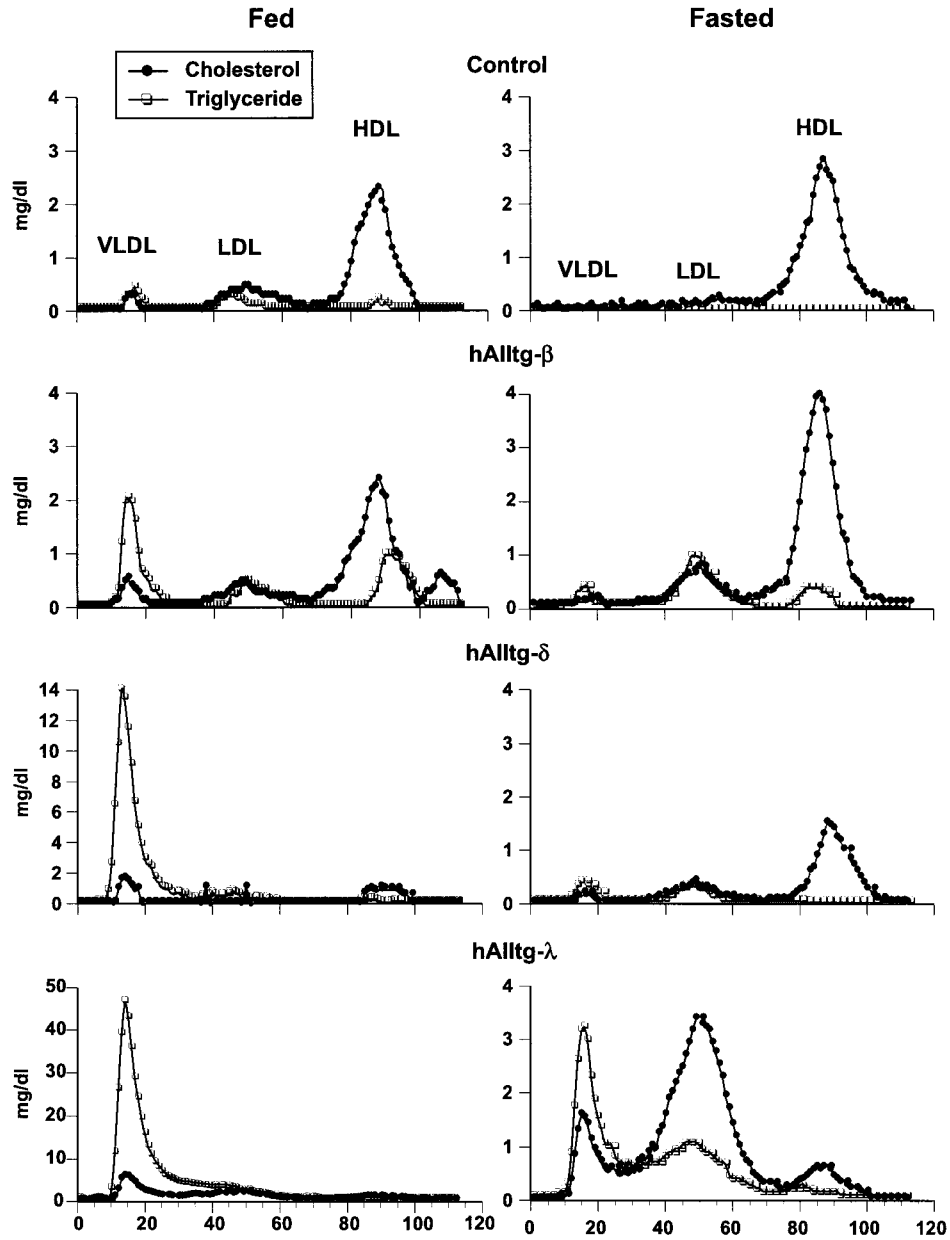

FIGURE 1: FPLC profiles of plasma lipoproteins from control and human apo A-II transgenic mice, either fed ad libitum or fasted overnight. FPLC chromatography was performed using two Superose 6 columns operating in series. Elution rate was 0.4 mL/min. Note the different scales for the lipoprotein profiles of fed δ and λ transgenic mice.

The Presence of Apo A-II in VLDL/LDL Is Related to the Plasma Concentration of Triglyceride-Rich Lipoproteins. Apo A-II was present in trace amounts in VLDL from fed β mice, but was not detected in VLDL from fasted animals (Tables 2 and 3). On the other hand, apo A-II was transported by apo B-containing lipoproteins in both δ and λ mice in the fed state but was clearly present only in VLDL (Tables 2−4) and remnants/LDL (not shown) in fasted λ mice. Thus, the presence of apo A-II in VLDL and LDL may be related to the plasma concentration of TG-rich lipoproteins, since most apo A-II disappears from the plasma compartment when animals are fasted rather than transferring onto HDL.

The HDL of β mice resembled HDL from control mice in apolipoprotein composition and carried mainly apo A-I, human apo A-II accounting for approximately 34% of total HDL protein (Table 4). Conversely, human apo A-II was the major HDL apolipoprotein in δ and λ mice, irrespective of nutritional state (Table 4). Interestingly, fasting increased the relative amounts of apo A-I in HDL from transgenic mice. This finding may result from the concomitant decrease of human apo A-II (Table 3), so that apo A-I was probably less displaced by apo A-II from the surface of HDL.

HDL Characterization by Analytical Ultracentrifugation. As shown in Figure 2, differences in HDL distributions between control and transgenic mice were both qualitative and quantitative. HDL from control and δ mice exhibited essentially unimodal distributions, although a shoulder could be detected on the denser side in the case of fasted δ mice. This distribution was shifted toward lower F1.21 values (and thus toward denser particles) in the transgenic animals. This holds true both in fasted and in fed mice, with peak *F* rates approximately 4.0 (controls) and 2.7 (δ) in the former, and 3.4 (controls) and 2.6 (δ) in the latter, suggesting a shift toward larger particles upon fasting. Fasted and fed λ mice presented with a plateau-shaped distribution over approximately the same *F* rate interval as that noted in the corre-

Table 1: Plasma Lipid Levels in Control and Human Apo A-II Transgenic Mice[a]

nutritional status	genotype	TG (mM)	TC (mM)	FC (mM)	PL (mM)	CE/TC (%)	human apo A-II (mg/dL)
fed	controls	0.41 ± 0.07	1.36 ± 0.07	0.31 ± 0.04	0.61 ± 0.21	77.8 ± 2.1	–
	hAIItg-β	0.97 ± 0.15	1.75 ± 0.15	0.39 ± 0.05	0.81 ± 0.05	77.7 ± 3.4	20.1 ± 2.5
	hAIItg-δ	9.51 ± 2.74[b]	3.14 ± 0.81	1.80 ± 0.60	2.18 ± 0.68	49.3 ± 5.3[d,f]	47.3 ± 6.3
	hAIItg-λ	16.02 ± 1.71[d,f]	4.83 ± 0.66[c]	3.12 ± 0.46[c,e]	3.05 ± 0.40[b,e]	35.8 ± 2.3[d,g]	66.7 ± 9.8[f]
fasted	controls	0.51 ± 0.12	1.66 ± 0.08	0.31 ± 0.02	1.34 ± 0.09	81.3 ± 2.0	–
	hAIItg-β	0.42 ± 0.11	1.07 ± 0.20	0.21 ± 0.05	0.71 ± 0.11	80.4 ± 3.1	18.2 ± 1.5
	hAIItg-δ	1.43 ± 0.16	1.77 ± 0.28	0.54 ± 0.12	1.02 ± 0.25	70.1 ± 2.2[j]	36.3 ± 4.2[f]
	hAIItg-λ	4.57 ± 0.66[d,f,i,k]	2.92 ± 0.29	1.34 ± 0.16[d,g,i]	2.04 ± 0.19[f,h]	54.1 ± 4.4[d,f,h]	35.3 ± 2.9[f,j]

[a] Four distinct plasma pools were characterized for each genotype, in the fed and fasted states. Results were expressed as mean ± SE. Statistical significance of the results was calculated by an unpaired *t*-test after analysis of variance. Abbreviations: TG, triglyceride; TC, total cholesterol; FC, free cholesterol; PL, phospholipid; CE, cholesterol ester. [b] $p < 0.05$ between transgenic and control mice in the same nutritional state. [c] $p < 0.01$ between transgenic and control mice in the same nutritional state. [d] $p < 0.001$ between transgenic and control mice in the same nutritional state. [e] $p < 0.5$ between transgenic line β and transgenic lines δ and λ. [f] $p < 0.01$ between transgenic line β and transgenic lines δ and λ. [g] $p < 0.001$ between transgenic line β and transgenic lines δ and λ. [h] $p < 0.05$ between transgenic lines δ and λ. [i] $p < 0.01$ between transgenic lines δ and λ. [j] $p < 0.05$ for the same genotype in the fed versus the fasted state. [k] $p < 0.01$ for the same genotype in the fed versus the fasted state.

Table 2: Lipoprotein Profile in Control and Human Apo A-II Transgenic Mice Fed ad Libitum[a]

genotype	lipoprotein	protein (mg/dL)	TG (mg/dL)	FC (mg/dL)	TC (mg/dL)	PL (mg/dL)	CE/TC (% mol)	human apo A-II (mg/dL)
controls	VLDL	4.8 ± 0.9	16.4 ± 2.2	1.33 ± 0.2	2.8 ± 0.1	3.5 ± 0.5	48.0 ± 2.6	–
	LDL	12.7 ± 3.5	6.1 ± 0.5	3.4 ± 0.5	11.4 ± 2.2	8.8 ± 1.3	68.4 ± 1.6	–
	HDL	73.7 ± 11.3	1.4 ± 1.0	4.3 ± 0.3	27.6 ± 1.7	35.5 ± 3.4	84.5 ± 1.2	–
hAIItg-β	VLDL	5.1 ± 0.3	27.1 ± 7.4	2.2 ± 0.3	4.3 ± 0.1	7.5 ± 0.1	49.2 ± 4.9	0.43 ± 0.1
	LDL	5.4 ± 1.2	5.6 ± 1.5	1.9 ± 0.5	5.6 ± 1.4	4.6 ± 1.3	65.8 ± 0.8	–
	HDL	84.4 ± 2.4	4.7 ± 2.3	4.2 ± 0.2	26.7 ± 1.3	35.7 ± 1.2	84.1 ± 1.4	26.5 ± 4.3
hAIItg-δ	CM	ND	72.2 ± 40.2	5.1 ± 3.3	5.3 ± 2.8	11.2 ± 5.2	19.1 ± 0.95	2.66 ± 0.6
	VLDL	39.2 ± 18.1	318.4 ± 119.2	31.9 ± 22.8	49.2 ± 22.6	81.9 ± 44.2	43.2 ± 0.82	16.5 ± 10.5
	LDL	23.1 ± 12.9	32.9 ± 12.2	7.9 ± 1.7	22.9 ± 8.7	22.9 ± 6.7	66.2 ± 5.2	3.7 ± 2.2
	HDL	43.1 ± 3.6	2.9 ± 2.8	1.9 ± 0.3[c,f]	9.7 ± 1.6[d,g]	15.8 ± 2.9[c,f]	80.2 ± 3.0	32.7 ± 5.3
hAIItg-λ	CM	ND	299.8 ± 165.5	20.2 ± 14.0	21.4 ± 11.9	48.0 ± 29.9	12.5 ± 6.2	13.9 ± 10.9
	VLDL	79.8 ± 14.6[c,e]	768.6 ± 174.0[c,f]	51.5 ± 12.4	91.8 ± 17.8[b,e]	150.4 ± 30.4[b,e]	37.1 ± 1.2	26.0 ± 6.8
	LDL	40.3 ± 9.2[e]	140.8 ± 54.7[b,e]	19.0 ± 3.1[c,f,h]	43.2 ± 7.3[b,e]	54.8 ± 7.3[c,f,h]	58.0 ± 1.6[b]	9.9 ± 3.2
	HDL	26.8 ± 9.1[b,f]	4.8 ± 1.8	1.2 ± 0.3[d,f]	5.2 ± 1.3[d,g]	9.6 ± 3.3[d,g]	80.0 ± 4.0	12.8 ± 3.1[h]

[a] Lipoproteins were isolated by sequential ultracentrifugation from four separate plasma pools obtained between 9.30 a.m. and 12.30 p.m. from mice fed ad libitum. Statistical significance of the results as in Table 1. Abbreviations: CM, chylomicrons; ND, not determined. Other abbreviations are as in Table 1. [b] $p < 0.05$ between transgenic and control mice in the same nutritional state. [c] $p < 0.01$ between transgenic and control mice in the same nutritional state. [d] $p < 0.001$ between transgenic and control mice in the same nutritional state. [e] $p < 0.5$ between transgenic line β and transgenic lines δ and λ. [f] $p < 0.01$ between transgenic line β and transgenic lines δ and λ. [g] $p < 0.001$ between transgenic line β and transgenic lines δ and λ. [h] $p < 0.05$ between transgenic lines δ and λ.

sponding δ mice; consequently, no peak *F* rate could be calculated. From a quantitative point of view, the observed differences among the three groups of mice were equally considerable, with HDL concentrations decreasing in parallel with the increase in expression level of the human apo A-II transgene. Indeed, the following apo A-II concentrations could be calculated for fasted animals: 203.6, 96.2, and 27.7 mg/dL in control, δ, and λ mice, respectively, and for fed animals: 84.5, 24.7, and <10 mg/dL in control, δ, and λ mice, respectively. Thus, HDL consistently increased about 3-fold after the overnight fast in all genotypes, the greatest relative increase being observed in fasted δ mice.

Distribution of Apo A-I and Apo A-II in HDL Subpopulations. We previously showed that HDL from control mice consisted of one homogeneous population of large-sized particles, whereas HDL from transgenic mice comprised an additional smaller-sized population (*13*). To determine the distribution of apo A-I and human apo A-II in HDL particles of different sizes, we subjected total HDL to two-dimensional electrophoresis. In control mice, large α HDL contained predominantly apo A-I (Figure 3A) with a small proportion (<30%) of murine apo A-II (Figure 3B). Large α HDL from β mice were poorer in apo A-I than the corresponding particles in control mice (Figure 3A), but contained a substantial amount of human apo A-II, in both large and small HDL particles (Figure 3B). In HDL from δ and λ mice, the apo A-I content of large HDL progressively decreased relative to that in the corresponding particles from control mice (Figure 3A), the amount of human apo A-II increasing in all three HDL particle subpopulations (Figure 3B). Apo A-I was mainly present in the larger HDL of all three transgenic lines. Figure 3C shows patterns of HDL from control and transgenic mice electrophoresed in parallel with HMW standards to evaluate particle size.

An interesting new finding in this study was the presence of pre-β migrating HDL containing human apo A-II in λ mice, whereas apo A-I-containing pre-β HDL could not be detected in these mice (Figure 3B). Pre-β HDL containing human apo A-II were also faintly detected in δ mice. On the other hand, small amounts of pre-β HDL with apo A-I were present in control, β, and δ mice.

Pre-β HDL Contain Human Apo A-II in Transgenic Mice. To confirm the presence of pre-β HDL containing human apo A-II in transgenic mice, eight HDL fractions were

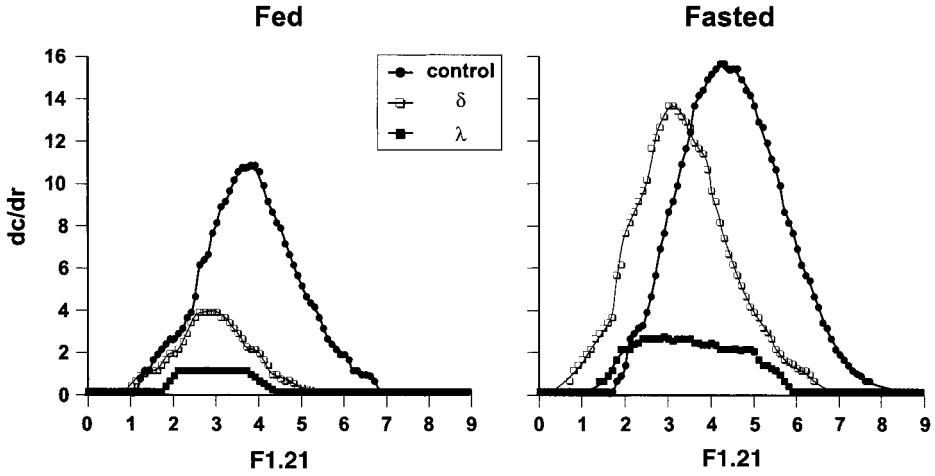

FIGURE 2: HDL profiles obtained by analytical ultracentrifugation. Experimental conditions were 52 000 rpm, 26 °C, density 1.21 g/mL, refractometric mode at 550 nm. Units of the *x* axis are flotation rates (*F*) determined at a density of 1.21 g/mL. The hydrated density of lipoprotein particles decreases with increasing flotation rates; i.e., in the figure, higher density HDL particles correspond to the left part of the diagram. The distributions presented were calculated from scans taken 70 min after the beginning of the run (up-to-speed time, approximately 16 min).

Table 3: Lipoprotein Profile in Control and Human Apo A-II Transgenic Mice Fasted Overnight[a]

genotype	lipoprotein	protein (mg/dL)	TG (mg/dL)	FC (mg/dL)	TC (mg/dL)	PL (mg/dL)	CE/TC (% mol)	human apo A-II (mg/dL)
controls	VLDL	2.3 ± 0.29	19.6 ± 2.2	1.3 ± 0.17	2.0 ± 0.32	3.8 ± 0.42	32.7 ± 2.7	–
	LDL	8.8 ± 2.3	15.3 ± 2.7	3.0 ± 0.52	7.1 ± 0.75	7.5 ± 1.4	58.6 ± 3.5	–
	HDL	102.7 ± 23.9	ND	7.4 ± 1.3[k]	45.2 ± 0.38[l]	67.2 ± 10.7[l]	79.6 ± 2.7	–
hAIItg-β	VLDL	1.8 ± 0.4	6.5 ± 0.1	0.5 ± 0.2	0.9 ± 0.3	1.6 ± 0.5	45.2 ± 2.4	–
	LDL	6.4 ± 0.1	8.3 ± 2.1	2.1 ± 1.0	5.0 ± 1.9	5.3 ± 2.5	61.2 ± 5.1	–
	HDL	120.4 ± 4.6	4.9 ± 2.1	6.0 ± 0.8	35.3 ± 0.1	51.0 ± 9.4	83.0 ± 2.4	20.9 ± 4.3
hAIItg-δ	VLDL	6.3 ± 0.38	35.0 ± 15.4	2.8 ± 1.1	6.5 ± 2.5	8.3 ± 3.4	60.3 ± 5.2[c,l]	–
	LDL	15.0 ± 1.7	22.4 ± 9.4	8.3 ± 2.2	21.0 ± 5.3	20.1 ± 5.5	61.3 ± 1.4	–
	HDL	68.4 ± 6.9	ND	4.3 ± 0.41	20.8 ± 1.8[c,e]	30.2 ± 1.4[c,k]	78.6 ± 3.9	29.1 ± 1.5
hAIItg-λ	VLDL	11.3 ± 0.08[c,e,k]	120.9 ± 55.1[l]	13.1 ± 0.90[d,g,j]	22.2 ± 1.5[d,g,j]	38.7 ± 3.8[g,j,k]	41.0 ± 1.9[h]	3.2 ± 0.9[k]
	LDL	46.4 ± 9.9[c,f,i]	56.7 ± 5.8[c,g,i]	21.2 ± 1.1[d,g,j]	53.1 ± 6.4[d,g,i]	59.3 ± 8.2[c,f,i]	59.2 ± 3.1	6.6 ± 1.6
	HDL	32.6 ± 11.6[b,f]	ND	3.4 ± 0.18[b]	11.2 ± 3.8[d,f]	18.8 ± 5.5[c,f]	77.2 ± 1.8	24.1 ± 6.1

[a] Lipoproteins were isolated by sequential ultracentrifugation from four separate plasma pools obtained between 9.30 a.m. and 12.30 p.m from mice fasted overnight. Statistical significance of the results as in Table 1. ND, not determined. Other abbreviations as in Table 1. [b] $p < 0.05$ between transgenic and control mice in the same nutritional state. [c] $p < 0.01$ between transgenic and control mice in the same nutritional state. [d] $p < 0.001$ between transgenic and control mice in the same nutritional state. [e] $p < 0.5$ between transgenic line β and transgenic lines δ and λ. [f] $p < 0.01$ between transgenic line β and transgenic lines δ and λ. [g] $p < 0.001$ between transgenic line β and transgenic lines δ and λ. [h] $p < 0.05$ between transgenic lines δ and λ. [i] $p < 0.01$ between transgenic lines δ and λ. [j] $p < 0.001$ between transgenic lines δ and λ. [k] $p < 0.05$ for the same genotype in the fasted state (present table) versus the fed state (Table 2). [l] $p < 0.01$ for the same genotype in the fasted state (present table) versus the fed state (Table 2).

prepared by density gradient ultracentrifugation. At all densities examined, control HDL consisted of homogeneous particles of approximately 10 nm diameter (Figure 4A). Expression of human apo A-II in β mice at the physiological level of 20 mg/dL resulted in the formation of a minor subpopulation of smaller HDL (around 7.8 nm), in accordance with previous studies (*13*, *17*, *18*). The concentration of these small HDL increased dose-dependently with the expression of human apo A-II, becoming the major HDL population in the higher-expressing λ mice. Apo A-I was the major HDL apolipoprotein in the lower-expressing β mice, the proportion of human apo A-II increasing in the denser, smaller HDL fractions (Figure 4B). On the other hand, human apo A-II was practically the sole HDL apolipoprotein in the higher-expressing λ mice, apo A-I being below detection level.

Figure 5 illustrates the distribution of apo A-I and A-II between α and pre-β HDL following two-dimensional electrophoresis of the larger and smaller HDL (fractions 1 and 8, respectively). HDL fraction 1 from control mice (as well as the other seven fractions, not shown here) carried apo A-I mainly in the large α-migrating HDL and a minor proportion in pre-β migrating HDL, whereas murine apo A-II was present in α HDL only (Figure 5A). Identical results were obtained in HDL fraction 1 of the lower-expressing β mice (not shown). HDL fraction 8 from β mice transported apo A-I in the larger-sized α HDL and a little in pre-β HDL, whereas α HDL of large and small sizes contained markedly greater amounts of human apo A-II (Figure 5B).

Interestingly, HDL fractions 1 and 8 of the higher-expressing λ mice comprised pre-β HDL containing human apo A-II (Figure 5C), without mouse apo A-I (not shown). Moreover, a substantial amount of pre-β HDL with human apo A-II was present in whole plasma from λ mice and a smaller amount in plasma from δ mice (not shown). Plasma from control and β mice contained only pre-β HDL with apo A-I (not shown).

Table 4: Apolipoprotein Composition of VLDL and HDL of Control and Human Apo A-II Transgenic Mice[a]

		VLDL					HDL
nutritional status	genotype	B100[b]	B48[b]	E[b]	hA-II[b]	C's[b]	% hA-II/mA-I[c]
fed	controls	36.1 ± 0.7	32.1 ± 2.4	25.5 ± 6.4	–	16.8 ± 2.4	–
	hAIItg-β	22.9 ± 1.1	23.4 ± 3.4	22.1 ± 0.5	9.9 ± 1.1	17.7 ± 2.1	34.7 ± 2.1
	hAIItg-δ	22.1 ± 5.1	27.2 ± 4.2	16.9 ± 1.4	17.5 ± 2.9	16.3 ± 2.5	87.5 ± 3.3[i]
	hAIItg-λ	18.7 ± 3.3	28.7 ± 1.1	14.1 ± 2.1	24.4 ± 1.9	14.1 ± 0.5[g]	98.5 ± 0.5[i,j]
fasted	controls	34.5 ± 3.1	26.1 ± 3.0	30.1 ± 1.7	–	11.3 ± 2.7	–
	hAIItg-β	26.4 ± 2.8	21.8 ± 0.6	24.6 ± 2.0	5.4 ± 0.5	16.9 ± 0.8	32.6 ± 3.4
	hAIItg-δ	25.8 ± 0.3[d]	28.6 ± 2.2	20.3 ± 1.5[e]	9.2 ± 2.4	18.8 ± 2.6	47.4 ± 1.6[h,m]
	hAIItg-λ	23.6 ± 1.3[e]	28.3 ± 1.9	17.1 ± 2.0[f]	14.1 ± 2.2	16.8 ± 2.0	84.5 ± 2.5[i,k,l]

[a] Apolipoproteins from four separate VLDL and HDL preparations were analyzed by SDS−PAGE and quantitated by densitometry. Results were expressed as mean ± SE Statistical significance of the results was calculated as in Table 1. [b] The percent composition of all VLDL apolipoproteins was obtained by 4−20% gradient SDS−PAGE electrophoresis. [c] The ratio of human (h) apo A-II to murine (m) apo A-I in HDL was obtained by 15% SDS−PAGE electrophoresis. [d] $p < 0.05$ between transgenic and control mice in the same nutritional state. [e] $p < 0.01$ between transgenic and control mice in the same nutritional state. [f] $p < 0.001$ between transgenic and control mice in the same nutritional state. [g] $p < 0.5$ between transgenic line β and transgenic lines δ and λ. [h] $p < 0.01$ between transgenic line β and transgenic lines δ and λ. [i] $p < 0.001$ between transgenic line β and transgenic lines δ and λ. [j] $p < 0.05$ between transgenic lines δ and λ. [k] $p < 0.001$ between transgenic lines δ and λ. [l] $p < 0.05$ for the same genotype in the fed versus the fasted state. [m] $p < 0.001$ for the same genotype in the fed versus the fasted state.

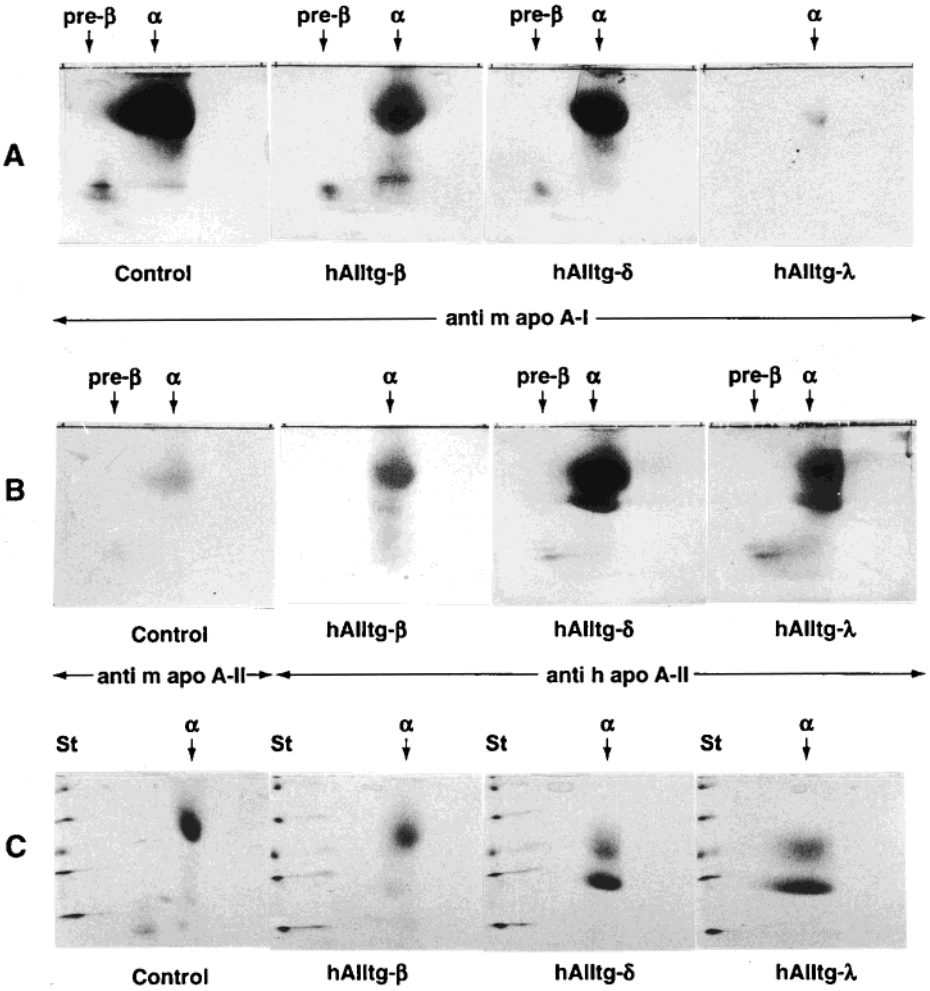

FIGURE 3: Immunoblotting of total HDL following two-dimensional electrophoresis. Total HDL were isolated by sequential ultracentrifugation at $d < 1.21$ g/mL, subjected to two-dimensional electrophoresis (14 μg of HDL protein/lane), and transferred to nitrocellulose membranes, as described under Experimental Procedures. (A) Immunoblotting with rabbit antiserum directed against mouse apo A-I (m apo A-I). (B) Immunoblotting with rabbit antiserum directed against mouse apo A-II (m apo A-II) or human apo A-II (h apo A-II), as specified. (C) Total HDL (8 μg of HDL protein/lane) were electrophoresed in agarose, and high molecular weight (HMW) standards (Pharmacia−Amersham) were added for the last 5 min of migration. The agarose strip containing both HDL and HMW standards was then electrophoresed in 8−25% Phast gels as described under Experimental Procedures and stained with Coomassie brilliant blue. St, HMW standards, the sizes of the markers being, from top to bottom, 13.3, 12.3, 10.2, 8.6, and 7.4 nm.

To determine whether pre-β migrating apo A-II was associated with lipids (and therefore functionally active), purified apo A-I, apo A-II, and HDL were subjected in parallel to two-dimensional electrophoresis. However, the free apolipoproteins migrated faster than HDL and could not be detected in the second dimension. Therefore, purified apo

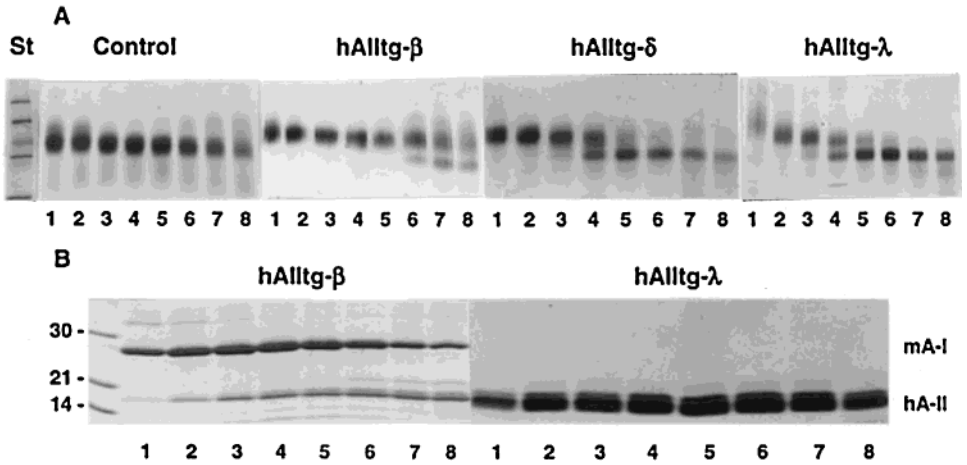

FIGURE 4: Characterization of HDL subfractions prepared by density gradient ultracentrifugation. (A) Nondenaturing gradient gel electrophoresis was performed on 4−20% gradient gels, and lipoproteins were stained with Coomassie brilliant blue. The same amounts of HDL protein from the 8 HDL fractions were electrophoresed (6 μg/lane). St, HMW standards, the sizes of the markers being, from top to bottom, 13.3, 12.3, 10.2, 8.6, and 7.4 nm. Fractions 1−8 correspond, respectively, to the following density intervals (g/mL): $1.072 < d < 1.081$; $1.081 < d < 1.091$; $1.091 < d < 1.101$; $1.101 < d < 1.112$; $1.112 < d < 1.121$; $1.121 < d < 1.133$; $1.133 < d < 1.145$; $1.145 < d < 1.156$. (B) SDS−PAGE of HDL apolipoproteins from hAIItg-β and hAIItg-λ mice was performed on 15% gels. On the left are the low molecular mass standards of, from top to bottom, 30, 21, and 14 kDa.

A-I and A-II were electrophoresed only for 5 min in agarose (as described under Experimental Procedures), and their electrophoretic patterns in the second dimension are shown on the left of each panel. This observation is an indirect argument that pre-β migrating apo A-II is in the form of pre-β HDL.

Figure 6 illustrates the respective concentrations of the eight HDL subfractions isolated by density gradient ultracentrifugation. In all groups of animals, fasting had a dual effect: it induced an increase in HDL concentrations and a shift in HDL particles to larger size, increased lipid content, and lower density. In all HDL fractions, the ratio of cholesteryl ester to protein was lower in transgenic compared with control mice, while the ratio of phospholipid to protein was higher (not shown).

Human Apo A-II Expression Induced a Deficit in LCAT Activity. LCAT activity was measured both with an exogenous substrate (allowing estimation of LCAT mass) and with an endogenous substrate (corresponding to the enzyme esterification activity with the endogenous lipoproteins). Under ad libitum feeding, LCAT activity against exogenous substrates was 18% and 34% lower in δ mice and λ mice, respectively, when compared with the corresponding controls. Such decreased activity was probably associated with the lower plasma HDL concentrations typical of our fed transgenic mice (Figure 7A). In the fasted state, LCAT activity levels were also significantly decreased. It is noteworthy that LCAT activity against endogenous substrates was significantly decreased in both δ and λ mice (by 70% and 86%, respectively, Figure 7B), as in other transgenic lines overexpressing human apo A-II (*18*). The dramatic decrease in LCAT activity occurred concomitantly with the very low apo A-I content of HDL. Interestingly, endogenous LCAT activity increased after an overnight fast, particularly in controls, probably reflecting the increase in HDL and apo A-I levels.

DISCUSSION

The mechanisms underlying the protective effects that HDL exert on atherosclerosis are not well established, in part because of incomplete knowledge of the formation and remodeling of HDL and of their interactions with TG-rich lipoproteins. Low plasma concentrations of HDL and apo A-I are not the only criteria defining an elevated cardiovascular risk, and factors such as the in vivo turnover rate and structural rearrangements of HDL play important roles. The present study focused on the metabolism of HDL and on the relationship between VLDL and HDL as affected in vivo by variations in the molar ratio of apo A-II/apo A-I in HDL particles and by the feeding−fasting transition. We showed that the increased level of expression of human apo A-II was a key determinant of the apo A-II/apo A-I ratio in HDL and modified in vivo the plasma concentration, particle size, and composition of HDL. We also showed that the decrease in VLDL during fasting was accompanied by an increase in HDL. We report for the first time the formation of pre-β HDL containing only human apo A-II in the high-expressing mice, in which apo A-I was nearly absent.

An important new finding of this study was the presence of pre-β HDL containing human apo A-II without endogenous apo A-I in the high-expressing transgenic mice. The rapid formation of pre-β HDL containing apo A-I upon incubation of fibroblasts with plasma has led to the proposal that pre-β HDL were the initial acceptor of cholesterol effluxing from cells (*8*). Pre-β HDL are generated by several mechanisms: incubation of HDL containing apo A-I or apo A-I and A-II, with LDL and cholesteryl ester transfer protein (CETP); HDL remodeling by hepatic lipase (HL) or phospholipid transfer protein (PLTP); and displacement of apo A-I by apo A-II in excess (*28*−*31*). Moreover, free apolipoproteins enhance cholesterol efflux from macrophages and generate pre-β HDL-like particles (*32*). Incubation of lipid-free apo A-II with either VLDL and LPL or LDL and oleate resulted in recovery of apo A-II in HDL particles with diameters of 7.8 and 7.4 nm and pre-β2 electrophoretic mobility (*33*).

The present study revealed a consistent increase in plasma HDL and a shift to larger-sized particles following an overnight fast, irrespective of genotype. To our knowledge, plasma HDL levels have not been compared in other studies

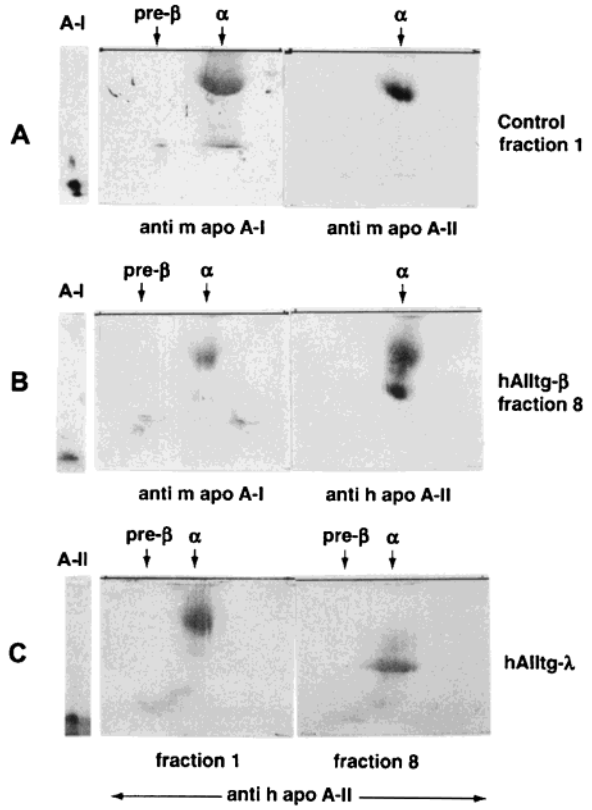

FIGURE 5: Immunoblotting following two-dimensional electrophoresis of HDL fractions obtained by density gradient ultracentrifugation. HDL fractions were the same as those in Figure 4. Characteristic fractions of HDL were selected on the basis of size for analysis of apo A-I and apo A-II contents. Constant HDL protein amounts (6 μg) were loaded on the gels. (A) HDL faction 1 from control mice probed with rabbit antisera directed against mouse apo A-I (m apo A-I, on the left) or mouse apo A-II (m apo A-II, on the right). (B) HDL fraction 8 from hAIItg-β mice probed with rabbit antisera directed against mouse apo A-I (m apo A-I, on the left) or human apo A-II (h apo A-II, on the right). (C) HDL fractions 1 and 8 from hAIItg-λ mice probed with rabbit antisera directed against human apo A-II (h apo A-II). Purified apo A-I and apo A-II were first electrophoresed for 5 min in agarose and then in 8−25% Phast gels as described under Experimental Procedures. The migration of mouse apo A-I is shown on the left of panels A and B and that of human apo A-II on the left of panel C.

under fed and fasted conditions. HDL are formed through association of cell-derived cholesterol and phospholipid with free apolipoproteins upon contact with peripheral tissues and acquire in the circulation surface components of TG-rich lipoproteins following hydrolysis of their TG by LPL (*12*). The former pathway is rate-limiting, as evidenced by the absence of HDL in Tangier patients and mice deficient in ATP binding cassette transporter 1 (ABCA1), which mediates efflux of cellular cholesterol and phospholipid (*34*, *35*). On the other hand, the drastic decrease in plasma HDL in PLTP knockout mice argues for an important contribution of the PLTP-mediated transfer of lipid surface components from VLDL to HDL (*36*). In all apo A-II transgenic lines, the rise in HDL occurred concomitantly with the marked reduction in TG-rich lipoproteins. Thus, the decrease in plasma VLDL and human apo A-II levels during fasting suggests efficient VLDL-TG hydrolysis by LPL and transfer of VLDL surface components onto HDL. The same mechanism may be operative in control animals, even though VLDL are low in the fed state due to efficient action of LPL. On the other hand, glucagon secretion is higher in fasted mice, and cyclic AMP may increase in tissues, stimulating ABCA1 expression and cholesterol and phospholipid efflux from peripheral tissues. Thus, the feeding−fasting transition illustrated the close reciprocal relationship between VLDL and HDL, at least in transgenic mice, and suggested that fasting may have additional effects consequent to hormonal changes.

The decrease in plasma apo A-I and HDL concentrations was consistently proportional to the level of expression of human apo A-II in our transgenic lines. The diminution in apo A-I level probably derives from its displacement from the surface of HDL in vivo by an excess of human apo A-II, as described earlier under in vitro conditions (*37*−*39*). Upon an increase of the physiological molar ratio of apo A-II/A−I from 1/2 to 1/1, 50% of apo A-I was displaced from reconstituted HDL, and essentially 100% of apo A-I was displaced at ratios greater than 2/1 (*37*). Under our experimental conditions in vivo, detached apo A-I was rapidly catabolized in the kidney (*40*), the major site of catabolism of lipid-poor apo A-I in rats (*41*) and humans (*42*). This finding concords with the greater reduction in plasma apo A-I (95%) than HDL, so that HDL of the high-expressing λ mice contained mainly human apo A-II and only traces of apo A-I. Thus, apo A-II is sufficient for the formation of HDL in the near-absence of apo A-I, consistent with the existence of HDL in apo A-I deficient mice (*43*). However, both apo A-I and apo A-II may be necessary for normalization of HDL metabolism and concentration.

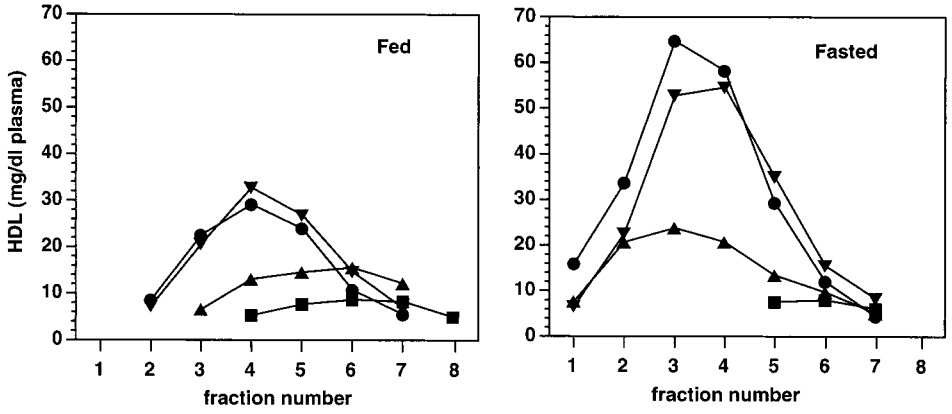

FIGURE 6: HDL subfractions obtained by density gradient ultracentrifugation. The HDL values represent the sum of all constituents. (A, left panel) Fed animals; (B, right panel) fasted animals. Control mice (●), hAIItg-β mice (▼), hAIItg-δ mice (▲), hAIItg-λ mice (■).

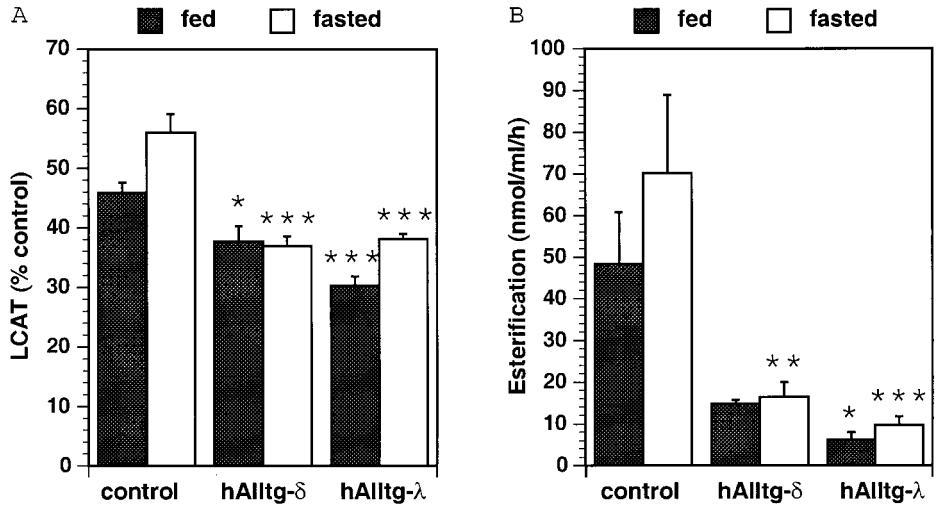

FIGURE 7: Plasma LCAT activity in control and transgenic mice. (A) LCAT activity measured in the presence of exogenous substrates, as an estimate of LCAT mass, and expressed as percent of control. (B) Endogenous LCAT activity, representing the cholesterol esterification rate in plasma, and expressed in nanomoles per milliliter per hour. Results are presented as mean ± SE, for 6 individual mice in each group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ for transgenic mice compared with control mice in the same nutritional condition.

Contrary to mice overexpressing human apo A-II, mice overexpressing mouse apo A-II displayed elevated plasma HDL with a high apo A-I content (*27*). Human apo A-II dose-dependently displaced human apo A-I in vitro (*37*−*39*) and mouse apo A-I in vivo (*13*, *18*) from the surface of HDL, whereas mouse apo A-II apparently did not (as is shown in Figure 8 of ref *27*). Displacement of apo A-I from HDL also occurred in mice overexpressing human monomeric apo A-II, indicating that the dimeric nature of human apo A-II is probably not the main cause of the differences in metabolic behavior between human and mouse apo A-II (*44*). Rather, structural differences may confer a greater hydrophobicity to human relative to mouse apo A-II.

An intriguing HDL modification in our A-II transgenic mice was the marked decrease in particle size. In control mice, endogenous apo A-I and A-II form homogeneous, large-sized HDL (*9*, *27*). Expression of human apo A-I and A-II in mice, either alone or in combination, yielded polydisperse HDL of sizes comparable with those of human HDL (*17*, *45*, *46*). In the present study, mouse apo A-I was consistently associated with the larger HDL particles, whereas high expression of human apo A-II resulted in HDL particles of smaller sizes, the smaller HDL containing essentially human apo A-II. Replacement of apo A-I by apo A-II under in vitro conditions did not alter HDL size (*28*, *47*), a finding which implies that the decrease in HDL size after apo A-I detachment in vivo might result from other metabolic changes induced by the increased molar ratio of human apo A-II/apo A-I.

The particle size and plasma concentration of HDL are modulated directly by the activities of HL and lecithin cholesterol acyltransferase (LCAT) and indirectly by LPL activity. HL overexpression in mice results in formation of smaller HDL (*48*). However, in our transgenic mice, human apo A-II impairs HL activity (*13*), indicating that modifications of HDL were probably not induced by HL. LCAT, activated by apo A-I, esterifies HDL-free cholesterol, which migrates to the core of HDL and increases particle size in the presence of LDL as a free cholesterol donor (*49*). Plasma HDL levels are very low both in LCAT-deficient states (*50*) and in cases of functional LCAT deficiency due either to the presence of apo A-I mutants, leading to less LCAT activation than for normal apo A-I (*51*, *52*), or to a low HDL-apo A-I content (present study and ref *18*). On the other hand, hydrolysis by LPL of the core TG of chylomicrons and VLDL is followed by transfer of their surface lipids to HDL (*15*, *16*). In humans, LPL activity is positively correlated with HDL levels, whereas hypertriglyceridemia due to LPL deficiency is associated with low levels of circulating HDL (*53*). The question now arises as to whether large HDL are transformed to smaller particles or whether small HDL are formed first and then do not mature to larger particles in mice overexpressing human apo A-II (*13*, *18*). Recent kinetic studies elegantly showed that small HDL containing two apo A-I molecules were converted in a unidirectional manner to medium- or large-sized HDL containing three or four apo A-I molecules per particle, respectively. The medium and large HDL were terminal particles in HDL metabolism, being catabolized primarily by the liver (*54*). We therefore suggest that the small apo A-II-rich HDL in our transgenic mice do not mature to larger particles as a result of the marked decrease in endogenous LCAT activity and the inhibition of LPL activity shown previously (*13*).

In conclusion, the overexpression of human apo A-II resulted in a great increase of the apo A-II/A-I ratio in HDL, dramatically affecting the metabolism of HDL and TG-rich lipoproteins, and inducing the formation of apo A-II containing pre-β HDL. An important question to be investigated in future studies is whether such pre-β HDL can act in vivo as cholesterol acceptors upon efflux from peripheral tissues. The feeding−fasting transition highlighted the reciprocal relationship between plasma HDL and VLDL levels and showed the impact of nutritional state on lipoprotein metabolism. Finally, our mouse models of human apo A-II overexpression will further understanding of the mechanisms which regulate HDL formation and remodeling.

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